AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph spanning pages 51, line 18 through page 53, line 2 with the following amended paragraph:

In yet other embodiments of the invention, overexpression and/or deletion *laeA* strains may be utilized in novel screens for new and useful secondary metabolite biosynthesis gene clusters. For example, using standard microarray technology now commonly employed in the field, one of skill in the art may construct a microarray containing, for example, nucleic acids representative of the expressed genes of wild-type *A. nidulans* (see, for example, D. Bowtell and J. Sambrook, DNA Microarrays: A Molecular Cloning Manual (2000) Cold Spring Harbor Laboratory Press and P. Baldi and G.W. Hatfield, DNA Microarrays and Gene Expression: From Experiments to Data Analysis and Modeling (2002) Cambridge University Press describing standard microarray techniques data analyses applicable in the present invention). The entire genome for *A. nidulans* has been sequenced and the sequence is available in annotated form for public use (see the Whitehead Institute/MIT Center for Genome Research website ~~http://www-genome/wi.mit.edu/annotataion/fungi/aspergillus/~~ www/genome/wi.mit.edu/annotataion/fungi/aspergillus/). Construction of the specific nucleic acids affixed to the array substrate may be based on, for example, an expressed sequence tag database provided by the University of Oklahoma (see ~~http://www.genome.ou.edu.fungal.html~~ www/genome.ou.edu.fungal.html). Using the microarray and standard hybridization techniques known in the field, the expression levels of genes in wild-type *A. nidulans* versus an *laeA* deletion mutant may then be compared to identify genes whose expression is reduced or absent in the *laeA* deletion mutant compared to the wild-type line. The artisan may subsequently examine the genomic sequence available for *A. nidulans* in order to identify putative secondary

metabolite biosynthesis cluster genes in the immediate vicinity of the relevant gene whose expression is initially identified as affected by the absence of *laeA* expression. As secondary metabolite biosynthesis genes are well known to occur in clustered fashion, as described in a plurality of references cited herein, new putative secondary metabolite gene clusters may be identified by this approach. Further, genes within a putative gene cluster may subsequently be disrupted and the mutant line's production of secondary metabolite products may then be compared with wild-type production in plus/minus fashion to identify the specific natural product produced by the newly-identified gene cluster. The natural product may then be isolated and characterized using standard techniques described and referenced herein.

Please replace the paragraph on page 53, lines 3-14, with the following amended paragraph:

It is envisioned the above-described screening strategies may be carried out not only between wild-type and *laeA* deletion mutants but also, and more preferably, between *laeA* overexpression mutants and *laeA* deletion mutants to obtain the greatest contrast in *laeA*-influenced secondary metabolite biosynthesis gene expression. As well, the screening methodology described herein is not limited to any one particular fungus but may be applied to any fungus having an *laeA* ortholog (e.g., *Aspergillus* other than *A. nidulans*). For example, the genome for *Fusarium graminearum* is now available and screens utilizing *laeA* overexpression or disruption strains to identify new *F. graminearum* secondary metabolite gene clusters may certainly be carried out based on the novel materials and teachings provided herein (also see Whitehead Institute/MIT Center for Genomic Research website at ~~http://www-~~

~~genome.wi.mit.edu/annotation/ fungi/fgi/~~ www-genome.wi.mit.edu/annotation/-fungi/fgi/ for *F. graminearum* genomic sequence).

Please replace the paragraph spanning page 53, line 15 to page 55, line 2, with the following amended paragraph:

In order to demonstrate the utility of the above-described screening methodology, the inventors carried out differential gene expression analyses with a microarray using ~~unigene~~ unique sequences available from an *A. nidulans* expressed sequence tag database (available through ~~throught~~ the University of Oklahoma website ~~http://www.genome.ou.edu.fungal.html~~ www/genome.ou.edu.fungal.html). In addition, approximately 145 gene sequences from GenBank that are not represented in the EST database were included, as well as the sequence for *laeA* (provided herein) The resulting array represented 6,529 unique gene sequences; this is a substantial portion of the approximately 10,000 expressed genes of the *A. nidulans* genome. Probe sequences of 24 base pairs were created and estimated to provide approximately 14 sequences per gene. These sequences were synthesized on chips by Nimblegen, Inc. (Madison, Wisconsin), using proprietary maskless technology. Total RNA was prepared from FGSC 26 (biA1; veA1) and RJW40.7 (*biA1*; *ΔlaeA::metG;veA1*) using TRIzol® reagent (Invitrogen, Carlsbad, CA) followed by RNeasy clean up (Qiagen Inc., Valencia, CA). The fungal strains used for this procedure are further detailed in Table 1 below. Total RNA was spiked with control RNA transcripts, converted to biotinylated cRNA and fragmented following the Affymetrix Expression Analysis Technical Manual (rev 1). Hybridization mixtures were prepared according to the array manufacturer's standard protocol using 10 mg biotinylated cRNA . Samples were incubated with the arrays overnight at 42°C. Chips were washed, stained

with streptavadin-linked Cy3 dye, and dried according to the manufacturer's protocol. Chips were scanned using a GenePix scanner (Axon Instruments, Union City, CA). The data were converted to a Microsoft Access database and normalized by the RNA spike control signals. Genes dependent on LaeA for expression were determined by expression ratios (wild type to mutant deleted *laeA* strain). Among the lowests ratios were genes known to be involved in penicillin and sterigmatocystin biosynthesis, consistent with other experimental data. Two additional LaeA-dependent genes were found to be adjacent to each other in the *A. nidulans* genome sequence (now annotated as AN8439.1 and AN8440.1). These genes are within 10 kb of genes encoding a non-ribosomal peptide synthase (AN8433.1), a tyrosinase (AN8435.1), and a P450 monooxygenase (AN8437.1), enzymes that are hallmarks of secondary metabolic pathways. Thus, the method according to the present invention was useful in the initial step of identifying a putative secondary metabolite biosynthesis gene cluster. This putative cluster is now available to be further characterized and defined using standard methodologies.

Please replace the paragraph on page 60, lines 2-18, with the following amended paragraph:

The *A. nidulans* aflR expression mutant, RYJ8 (derived from MRB300, see Supplementary Information), was transformed with an *A. nidulans* genomic cosmid library. Norsolorinic acid (NOR) producing transformants were purified and a cosmid, pCOSJW3, that complemented the mutation was rescued from one transformant. NOR is a visible precursor in the ST biosynthetic pathway and commonly used as an indicator of ST production[5]. pJW15, a 4.5 kb KpnI-EcoRI subclone of pCOSJW3 also complemented the mutation and was sequenced using synthetic primers and ABI PRISM DNA sequencing kit (PerkinElmer Life Science). The

mutant allele, *laeA1*, was sequenced after subcloning a 3 kb PCR fragment from RYJ8 genomic DNA amplified with primers LAE1 and LAE2 (see Supplementary Information) into Zero Blunt TOPO vector (Invitrogen Co.) to produce pJW31. RACE technology using Gene Racer Kit (Invitrogen Co.) was employed to clone laeA cDNA according to manufacturer's instruction. The cloned cDNA was then sequenced. The Institute for Genomic Research (TIGR) contains partial *A. fumigatus* genome sequence (~~http://www.tigr.org/tdb/e2k1/afu1/~~ www/tigr.org/tdb/e2k1/afu1/). A putative *A. fumigatus laeA* homolog was obtained by blasting the *A. fumigatus* data with the *A. nidulans laeA* sequence.

Please replace the paragraph spanning pages 67, line 6 through page 68, line 8, with the following amended paragraph:

Previously a mutagenesis screen led to isolation of 23 mutants displaying loss of ST production but normal sporulation in *A. nidulans*[5]. Three of the mutants were unable to express aflR that encodes a ST cluster Zn_2Cys_6 transcription factor regulating ST biosynthetic gene expression[6]. The inventors were able to complement one of these three mutants, RYJ8, with an *A. nidulans trpC* genomic cosmid library. Sequencing of a 4.5 kb subclone (pJW15) of the complementing cosmid pCOSJW3 revealed a 3 kb ORF designated as *laeA* (for loss of *aflR* expression). Sequencing of the mutant allele, *laeA1*, from RYJ8 showed it has a base pair transversion (1455; C -> G) and a one base pair deletion (1453 bp) of the gene. The deletion resulted in a premature stop codon. Examination of genomic and cDNA sequence revealed that *laeA* has one intron and three putative AflR binding sites[6], one in the promoter (-607) and two in the encoding region (607 and 1487, Fig. 1a). cDNA analysis showed *laeA* possesses a 5' untranslated region (642 bp) (Fig. 1a). Analysis of available genomic databases indicated only

filamentous fungi have obvious LaeA homologs including *A. fumigatus* (human pathogen, aspergillosis, TIGR ~~http://www.tigr.org/tdb/e2k1/afu1/~~ www/tigr.org/tdb/e2k1/afu1/), *Neurospora crassa* (model fungus, GenBank™), *Magnaporthe grisea* (plant pathogen, rice blast fungus, ~~http://www-genome.wi.mit.edu/annotation/fungi/magnaporthe~~ www-genome.wi.mit.edu/annotation/fungi/magnaporthe), *Coccidioides immitis* (human pathogen, coccidioidomycosis, GenBank™) and *Fusarium sporotrichioides* (plant pathogen, trichothecene mycotoxin producer, www/genome.ou.edu/fsporo.html) (Fig. 1b). Examination of the LaeA amino acid sequence (375 amino acids) revealed a conserved s-adenosylmethionine (SAM) binding site found exclusively in nuclear protein methyltransferases (Fig. 1b)[7]. Although the amino acid sequence of LaeA did not show the presence of a nuclear localization motif, green fluorescent protein (sGFP) tagging to either the 5' or the 3' end of *A. nidulans laeA* showed LaeA to be localized in the nucleus (Fig. 5). Biochemical analysis showed that LaeA methylated a ca. 30 kDa nuclear protein (Fig. 1c).

Please replace Table 2, on page 64, lines 1-24, listing the sequence of primers, with the following amended Table 2:

Table 2. Primers

Primer	Sequence[a]	Restriction sites	SEQ ID NO:
LAE1	ATCTACCTTTCTGGGCTCCTGG		4
LAE2	CGTGAAGAACTTGGCGTTGTAG		5
LA2	GACGAGCTCGTGGAACAGTGGAAGGAAC	*Sac*I	6
LA3	GCGAAGCTTATGAACCGCATCAACCGA	*Hind*III	7
OEF	GCTGTGAAGCTTTGTACCCTGTTTCGCC	*Hind*III	8
OER	GATTTGAAGCTTTGCTGGCATGGAACGG	*Hind*III	9
MT1	ATGCTGAAGCTTGGAAACTGGGAAAGGGGTC	*Hind*III	10
GFP2	TGACGAATTCTCTTAATGGTTTCCTAGCCTG	*EcoR*I	11
GFP31	TGCGGAATTCATGAGCAAGGGCGAGGAA	*EcoR*I	12
GFP4	GGATGCCTCGAGTTTGTACAGCTCGTCCATGC	*Xho*I	13
GFP5	AAGCAGCTCGAGTAAGAGCAAAAGGCGACCAC	*Xho*I	14
GF1	CTAGCGAAGCTTGCCACCATGAGCAAGGGCG	*Hind*III	15
GF2	CGGCGAATTCCTTGTACAGCTCGTCCATGC	*EcoR*I	16
GF3	TTTGGAATTCGTTTCGCCGCTGATGTTTGAG	*EcoR*I	17
FUM1	GCGCACTTCTTTGTTTTCCCCT		18
FUM2	CATCGGAATTCTTTCTTGAGCGGCC	*EcoR*I	19
FUM3	TACCAGGATCCAAAACCTCTCGCCA	*BamH*I	20
FUM4	CATGACGGTAACTAAGGATTTGG		21

[a] underlined sequences show placement of restriction sites shown on the right.